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Partitioning pattern of carbon flux in a *Kobresia* grassland on the Qinghai-Tibetan Plateau revealed by field ^{13}C pulse-labeling

YIBO WU*, HONGCHAO TAN*, YONGCUI DENG*, JING WU*, XINGLIANG XU†, YANFEN WANG*, YANHONG TANG‡, TERUO HIGASHI§ and XIAOYONG CUI*

*College of Resources and Environment, Graduate University of Chinese Academy Sciences, Beijing 100049, China, †Institute of Geographical Sciences and Natural Resources Research, the Chinese Academy of Sciences, 11A Datunlu, Beijing 100101, PR China, ‡National Institute for Environment Studies, 16-3 Onogawa, Tsukuba, Ibaraki 305-8569, Japan, §Institute of Applied Biochemistry, University of Tsukuba, 1-1-1 Tennodai, Tsukuba, Ibaraki 305-8577, Japan

Abstract

Characterizing the carbon turnover in terrestrial ecosystems is critical for understanding and predicting carbon dynamics in ecosystems. We used *in situ* ^{13}C pulse labeling to track photosynthetic carbon fluxes from shoot to roots and to soil in a *Kobresia humilis* meadow on the Qinghai-Tibet Plateau. We found that about 36.7% of labeled carbon was translocated out from the shoots within the first 24 h after photosynthetic uptake. This is equivalent to 66.1% of total ^{13}C moving out from the shoot during the 32-day chase period, indicating a rapid and large translocation of newly fixed carbon to belowground parts in these alpine plants. 58.7% of the assimilated ^{13}C was transferred belowground. At the end of the chase phase, 30.9% was retained in living roots, 3.4% in dead roots, 17.2% lost as belowground respiration and 7.3% remained in the soil. In the four carbon pools (i.e., shoots, living roots, dead roots, and soil pools), living roots consistently had the highest proportion of ^{13}C in the plant–soil system during the 32 days. Based on the ^{13}C partitioning pattern and biomass production, we estimate a total of 4930 kg C ha^{-1} was allocated belowground during the vegetation growth season in this alpine meadow. Of this, roots accumulated 2868 kg C ha^{-1} and soils accumulated 613 kg C ha^{-1}. This study suggests that carbon storage in belowground carbon pools plays the most important role in carbon cycles in the alpine meadow.

Keywords: ^{13}C pulse labeling, alpine meadow, carbon allocation, carbon turnover, grazing

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Introduction

Carbon allocation among different C pools is crucial in determining carbon balance at various scales from ecosystem to global scales (Rice *et al*., 2004; Luo *et al*., 2009). In terrestrial ecosystems, plants fix CO_2 from the atmosphere and a significant proportion of fixed carbon is transferred and accumulated in the belowground carbon pool, including roots and soil organic matter. Belowground carbon storage is particularly significant in grassland ecosystems. A small change in belowground carbon pools could have a large impact on atmospheric CO_2 concentration because belowground carbon storage is more than twice the size of the atmospheric carbon pool (Schlesinger, 1990). However, carbon allocation from shoots to roots and further to the soil, has been poorly quantified in the field at the ecosystem scale. Our current knowledge of carbon translocation in terrestrial ecosystems is mostly based on indirect estimates from inventories of carbon stocks in different carbon pools, such as the plants, plant litter and soil (Fang *et al*., 2001; Schuur *et al*., 2001; Tao *et al*., 2006). However, measurements of the size of carbon pools cannot adequately describe *in situ* ecosystem carbon turnover (Williams *et al*., 2005; Heimann & Reichstein, 2008). Thus, there is an urgent need for increased information on *in situ* carbon acquisition and translocation at the ecosystem scale.

Isotope tracer technology offers a suitable approach for tracking and quantifying carbon flows in terrestrial ecosystems *in situ*. The rates and quantities of ^{13}C flux from shoots after pulse-labeling to belowground allocation can be used to quantify the contribution of recently fixed plant photosynthate to ecosystem carbon cycling

Correspondence: Xiaoyong Cui, e-mail: cuixy@gucas.ac.cn

over a given time span. Using this approach, partitioning of assimilated carbon has been documented in terms of translocation to roots (Ostel *et al*., 2000; Johnson *et al*., 2002b; Butler *et al*., 2004), and to soil (Domanski *et al*., 2001; Johnson *et al*., 2002a; Dilkes *et al*., 2004), and others have analyzed the influence of climate conditions on partitioning (Mehrag & Killham, 1989, 1990; Martin & Merckx, 1992; Kessel *et al*., 2000; Rangel-Castro *et al*., 2004; Hill *et al*., 2007). The translocation of carbon into different carbon pools can vary greatly in different plants and ecosystems. For example, crops seldom transfer more than 33% of assimilated carbon to the soil (Kuzyakov, 2001). Some studies on meadow plants show that the fraction of assimilated carbon allocated belowground ranged from 28% to 48% (Saggar *et al*., 1997; Kuzyakov *et al*., 1999; Domanski *et al*., 2001). Partitioning can also differ in different growth stages of plants (Mehrag & Killham, 1990; Swinnen *et al*., 1994; Grayston *et al*., 1997), vary under different nutrient conditions (Saggar *et al*., 1997; Kuzyakov, 2001; Kuzyakov *et al*., 2002), and change under different environmental conditions (Saggar *et al*., 1999; Staddon *et al*., 2003; Rangel-Castro *et al*., 2004). Carbon translocation in perennial grasses is limited to controlled laboratory pot experiments with single plant species such as *Lolium perenne* and *Bromus erectus* (Warembourg & Estelrich, 2000; Kuzyakov, 2001; Butler *et al*., 2004), and a few upland grasslands (Johnson *et al*., 2002a; Staddon *et al*., 2003; Rangel-Castro *et al*., 2004; Leake *et al*., 2006; Kaštovská & Šantrùèková, 2007). There has been much less documentation for plants in alpine ecosystems where plants are often perennial and differ greatly in many respects from most other crops. For example, plant belowground matter in alpine meadows can account for 80% of total biomass, a much higher proportion than in crops (Whipps, 1990). These differences in plants may result in large difference in carbon partitioning both in plants and within the ecosystem.

Natural grasslands, including tundra, covers about 30% of the Earth's surface and contains 452.3 Pg C (nearly 1/4 of total global stocks) in both plant biomass and soil (Adams *et al*., 1990). Alpine meadow is the most widely distributed vegetation on the vast Qinghai-Tibet Plateau in western China. The meadow ecosystem appears to play the most important role in both uptake and storage of carbon on the Plateau (Kato *et al*., 2006; Yang *et al*., 2008). Within China and East Asia, the Plateau has been affected very early on by climate change (Li & Tang, 1988). Ecosystems on the Plateau are therefore thought to be fragile and sensitive to climate change. Thus, understanding allocation patterns of recently assimilated carbon in alpine meadow can provide important insights into carbon cycles and its ecological effects as well as feedbacks to global climate change. In this study, we conducted a $^{13}CO_2$ pulse labeling experiment in the field. Previous studies showed that plants had a higher root/shoot biomass ratio at lower soil or growth temperature (Lambers *et al*., 1998; Hui & Jackson, 2005; Mokany *et al*., 2006; Yang *et al*., 2009). We hypothesized that vegetation allocated a higher proportion of newly fixed carbon to belowground biomass and soil pools in alpine ecosystems on Qinghai-Tibetan Plateau. The purpose of this study was: (1) to quantify the partitioning of recently fixed carbon among plant and soil carbon pools; and (2) to estimate the net transport of carbon from plants to soils in an alpine meadow on the Qinghai-Tibet Plateau under field conditions.

Materials and methods

Study site

The field site was an alpine *Kobresia humilis* meadow (37°29–45′N, 101°12–23′E) at the northeast edge of the Qinghai-Tibet Plateau. The meadow was approximately 3250 m in elevation. The climate is dominated by the Southeast monsoon and the Siberian high-pressure system. It has a continental monsoon type climate, with severe and long winters and short, cool summers. The average air temperature is −2 °C with an extreme maximum of 27.6 °C and an extreme minimum of −37.1 °C. Annual precipitation ranges from 426 to 860 mm, 80% of which falls in the short summer growing season from May to September. Annual average sunlight is 2462.7 h with 60.1% of total available sunshine. The soil was a clay loam with a mean thickness of 0.65 m, and was classified as Mat Cry-gelic Cambisols according to the Chinese National Soil Survey and Classification System (Chinese Soil Taxonomy Research Group in Institute of Soil Science of CAS, 2001). The top 5–10 cm of the soil was wet and rich in organic matter (52 ± 3 g kg^{-1}, measured during this experiment). The plant community was dominated mainly by three perennial sedges, *K. humilis* (C. A. Mey. Ex Trautv) Serg., *K. pygmaea* (C. B. Clarke) and *K. tibetica* Maximowicz (Cyperaceae). The subdominants included *Stipa aliena* Keng, *Elymus nutans* Griseb, *Festuca ovina* Linn, *Poa* spp. Linn, *Gentiana straminea* Maxim, and *Polygonum viviparum* Linn. Aboveground plant living biomass averaged 342 g m^{-2} for 1980–1993, and peaks in July or August, and then decreases rapidly in September (Li & Zhou, 1998). Root biomass in the top 20-cm soil layer was 2519 g m^{-2}, of which about 70% was in the 0–10 cm soil layer. Root biomass decreased markedly with the increase of soil depth below 20 cm (Tao *et al*., 2006). The study site was grazed by yaks and sheep in winter.

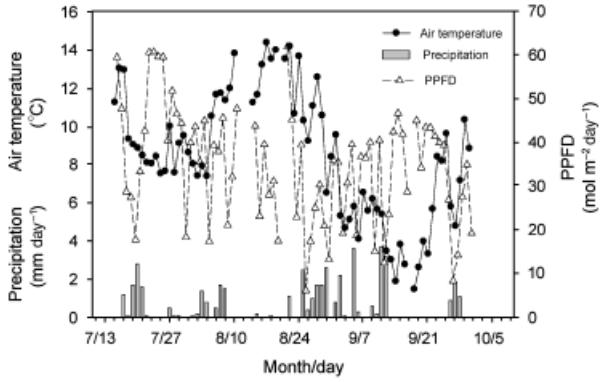

Fig. 1 Air temperature, radiation, and precipitation at the study site during the experiment from mid-July to the end of September in 2007.

We conducted the experiment under average weather conditions, with days dominated by clear skies during the period from July 20 to September 1, 2007. Air temperature, radiation, and precipitation were shown in Fig. 1 for this period of time. Pulse-labeling was carried out on July 29, which is near the middle of the growing season. The chase period for sampling lasted until the end of the vegetation growth period. Aboveground standing biomass measured on July 24 was 268 g m^{-2}, which was close to the average of annual values in the meadow.

^{13}C pulse labeling

We randomly set four replicate plots in the alpine meadow. The closest distance between two neighboring plots was around 5 m. We installed an open cylinder chamber in each plot in the late afternoon of the day before isotope labeling and sealed a transparent ceiling to the chamber just before labeling. The chambers had transparent PVC walls and an acrylic film ceiling. We inserted the chamber walls into the soil to about 5-cm depth and packed a nylon mesh with pore size of 45 μm. These meshes were extended to 10-cm depth so as to cut off roots from plants outside of the labeling chambers, while allowing nutrient and water exchange between the soil inside and outside of the chambers. Extra fine earth was packed firmly around the base of the chamber to reduce gas leakage. Each chamber was 1 m in diameter and 0.2 m in height. The inner surface of the chamber was smeared with anti-fog agent to reduce water vapor condensation during labeling, which can increase light intensity and reduce the $^{13}CO_2$ dissolved into water drops on the chamber's inner surfaces.

Pulse labeling was carried out at 11:00 hours in the morning on July 29, 2007, which was a clear day. ^{13}C-enriched CO_2 (99 at% ^{13}C) was stored in a high-pressure bottle and delivered to the chambers simultaneously through pressure reducing valves and 4-mm diameter inlet tubes in each individual chamber. Labeling started immediately after the ceilings were closed and tightly sealed. The flow rate was measured by flow meters and maintained at 0.125 L min^{-1} for 40 min. The total volume of CO_2 input was so small that it did not change air pressure inside the chambers. $^{13}CO_2$ supply was stopped and the chambers were maintained closed for another 5 h. During the labeling period, air inside each chamber was circulated by a vertically mounted electric (12V, 0.21A) fan in the center of each chamber. The flow rate and labeling time was determined according to photosynthetic rates and belowground productivity as previously measured, to ensure sufficiently higher ^{13}C abundance in both shoot and root samples compared with unlabeled control samples during the whole chase period. CO_2 concentration in the chambers was not measured during labeling. It would decrease quickly to a low level according to reported photosynthetic rate at both leaf and community levels (Shi *et al*., 2001; Cui *et al*., 2003; Kato *et al*., 2006). The chambers were removed upon completion of labeling.

Air sampling

Air samples were collected at 0, 1, 3, 6, 12, 24, 48, 96 h after labeling with opaque PVC chambers of two different sizes. The bigger chambers, 15 cm tall and 20 cm in diameter, were used to sample CO_2 respired by the plant–soil system. Aboveground vegetation was not removed in these chambers. The smaller chambers, 25 cm tall and 5 cm in diameter, were used to cover soil surface where aboveground plant parts were harvested. At the top of each chamber, there was a hole sealed by a rubber septum for sampling air inside with a syringe. From each chamber, 70 mL of air was collected slowly and injected into an aluminum–plastic composite gas bag (TPV-005, Dalian Delin Gas Packing Co., Ltd., Dalian, China) for ^{13}C analysis. At each sampling, atmospheric air samples were also gathered with a syringe at 2 m above the soil surface for ^{13}C control measurement.

Tissue and soil sampling

Shoot samples were collected when taking gas samples, and were also collected 8, 15, 22 and 32 days after labeling. Aboveground plant parts of all species were harvested and pooled as shoot samples by clipping at the soil surface before undertaking the soil sampling. Only green shoots were used for further ^{13}C analysis. Soil cores 5 cm in diameter were taken to 20 cm depth immediately after the gas sampling on the 1st, 2nd, 4th,

8th, 15th, 22nd, and 32nd days after labeling. All roots and soil in the cores were carefully extracted and sieved with a 2-mm screen. The soil samples that passed through the sieve were air-dried and stored at 4 °C for about 3 months before being analyzed for total C and ^{13}C. The sampled roots were carefully washed by wet sieving though a 0.5 mm screen to remove attached soil and dark-brown/black debris. The roots were further separated into living and dead components based on their color. The shoot and root samples were oven-dried at 70 °C for 48 h.

Carbon content and its isotope measurement

Carbon content and the $^{13}C/^{12}C$ ratio were measured with a MAT 253 stable isotope ratio mass spectrometer system (Thermo Fisher Scientific Inc., Bremen, Germany). Soil samples were prepared by washing in 0.1 mol L^{-1} HCl until no air bubbles appeared to remove all carbonates. The acid-treated samples were oven-dried at 105 °C for 24 h. They were then ground to homogeneously fine powders manually with a pestle and mortar. Shoot and root samples were also ground using a MM 200 steel ball mill (Retsch GmbH, Haan, Germany). The samples were packed in tin cups and combusted in an elemental analyzer (FlashEA1112 Series, Thermo Fisher Scientific Inc.). Via a variable open-split interface, the gases produced were led into the mass spectrometer, which was operated in continuous flow mode. Air samples were inserted into MAT 253 with gas bench II linked to a mass spectrometer. The precision for $\delta^{13}C$ analysis was $<0.1‰$.

Calculations and data analysis

Natural abundance of ^{13}C in samples was expressed as $\delta^{13}C$ ‰ units relative to Pee Dee Belemnite. In order to facilitate comparisons with other studies, we also calculated the enrichment values as ^{13}C at% excess, the increase in ^{13}C atoms due to pulse-labeling expressed as the percentage of total carbon atoms in the sample by the following equations:

$$R_{\text{sample}} = \left(\frac{\delta^{13}C}{1000} + 1\right) \times 0.011237, \quad (1)$$

$$^{13}\text{C}\,(\text{at}\%) = \left(\frac{R_{\text{sample}}}{R_{\text{sample}} + 1}\right) \times 100, \quad (2)$$

$$^{13}\text{C excess}\,(\text{at}\%) = {}^{13}\text{C of samples}\,(\text{at}\%) - {}^{13}\text{C of natural abundance}\,(\text{at}\%), \quad (3)$$

where R_{sample} is the isotope ratio of sample $^{13}C/^{12}C$, and 0.011237 is the ratio of $^{13}C/^{12}C$ in Pee Dee Belemnite. ^{13}C at% represents the percent of ^{13}C atom in total carbon atoms.

To estimate the amount of ^{13}C incorporated into various plant and soil pools the following equation was used:

$$^{13}\text{C amount}\left(\text{mg m}^{-2}\right) = {}^{13}\text{C excess}\,(\text{at}\%) \times \text{C pool size}\left(\text{g m}^{-2}\right) \times 10, \quad (4)$$

where carbon pool size is the carbon content in shoots, roots and soil, and was assumed to be constant during the whole chase period. Atmospheric background was corrected using ^{13}C at% excess instead of $\delta^{13}C$ as in the above equation.

Results

Plant carbon content and fixation of ^{13}C during the labeling period

The carbon content of shoots, living and dead roots was 44.3% ± 0.4%, 43.4% ± 0.7%, and 43.5% ± 0.8%, respectively, in the chase period. These measured values were used to calculate carbon flow as shown in Table 2 and Fig. 4.

During the period of about 6-h of labeling, about 3.69 g ^{13}C m^{-2} was introduced into each chamber, of which approximately 17.5% (i.e. 645.95 mg ^{13}C m^{-2}) was recovered from plant shoots immediately after labeling. This means that at that time shoot biomass contained almost 0.6% C labeled by ^{13}C, while a proportion of the newly fixed ^{13}C had already been released by respiration and translocated belowground.

Respiration loss of ^{13}C

The value of $\delta^{13}C$ in CO_2 from total respiration (both soil and shoot respiration) declined about 88.37%, from 1381.84‰ to 160.66‰, during the 96 h period after labeling. The decreased from soil respiration in CO_2 was 92.79%, from 1685.28‰ to 121.46‰. The CO_2 from soil respiration tended to have a higher $\delta^{13}C$ value than the CO_2 from total respiration in the first 3 h after labeling (Fig. 2). Thereafter, the CO_2 from the total respiration and from soil respiration showed a similar $\delta^{13}C$ value until the end of the 96 h chase time. A very high rate of $^{13}CO_2$ loss through respiration occurred in the first 12 h after labeling, i.e. during the night from 17:00 to 05:00 hours of the next day. This loss rate diminished exponentially within the next 84 h (Fig. 2).

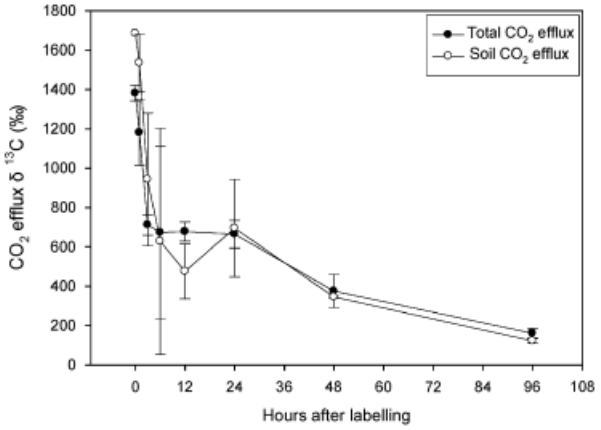

Fig. 2 Temporal variation of carbon isotope composition of CO_2 efflux by soil respiration and ecosystem respiration (including shoot respiration) during the first 96 h after labeling. Mean values ($n = 4$) and standard deviations are given.

^{13}C allocation within the plant–soil system

A large amount of $^{13}CO_2$ was detected in soil respiration immediately after labeling (Fig. 2), demonstrating a rapid and significant allocation of newly fixed carbon into belowground pools. This was confirmed by a marked increase of $\delta^{13}C$ values in root samples collected immediately after labeling compared with those from unlabeled control plots (Fig. 3). The $\delta^{13}C$ value 1 day after labeling increased about 479‰ in the shoots, 21.16‰ in living roots, and 9.06‰ in dead roots, but showed no significant change in the soil. The $\delta^{13}C$ of shoots declined exponentially over time and the decrease amounted to 50% within the first 4 days.

The amount of fixed ^{13}C remaining in the plant–soil system was relatively stable in the first 32 days, assuming that the carbon content of shoot, roots and soil were constant during this period of time (Table 1). The proportion of ^{13}C allocated to shoot and living roots gradually decreased, while that allocated to dead root and soil pools were low and steady (around 4% and 7%, respectively). The highest proportion of ^{13}C was always in the living roots, accounting for 47.9% on the first day and 30.9% on day 32. The amount of ^{13}C in the shoots decreased from 645.95 mg $^{13}C\,m^{-2}$ immediately after labeling to 287.56 mg $^{13}C\,m^{-2}$ on day 32, indicating a loss of about 55.5% of ^{13}C during the chase period. The ^{13}C allocation to roots continued to increase until it reached its maximum on day 15 after which it slowly decreased. The amount of newly fixed ^{13}C partitioned to soil slowly increased from day 1 until day 22, reaching its peak later than the peak in living roots. During the period from day 1 to day 32, the distribution of ^{13}C in dead roots was not significantly variable, and showed a smaller change compared with the change in living roots.

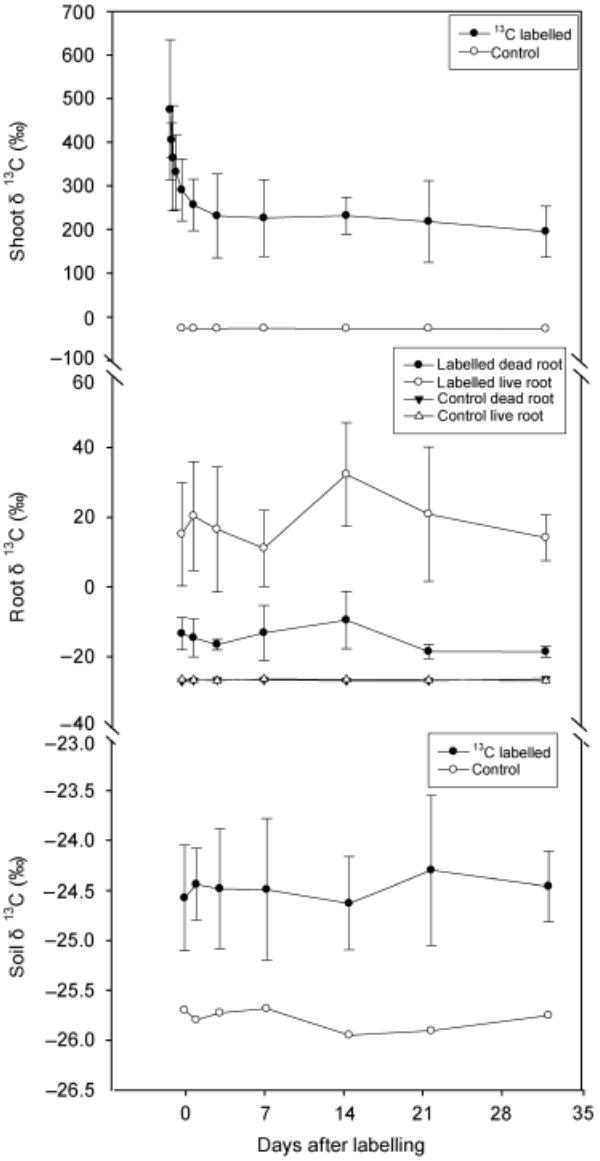

Fig. 3 Temporal variation of carbon isotope composition of shoots, roots and soil C sampled within the plant–soil system during the 32-day chase period. Mean values ($n = 4$) and standard deviations are given.

Discussion

Spatial variability in labeling and sampling

There was a large variability of ^{13}C among the four plots, especially in the root ^{13}C content (Fig. 3). Such spatial variability seems common in nature (Staddon

Table 1 Temporal changes of ^{13}C amount in the plant-soil system and ^{13}C distribution (in %) among different pools

Time (days)	^{13}C in the system (mg ^{13}C m^{-2})	Proportion of ^{13}C in individual pool (%)			
		Shoots	Living roots	Dead roots	Soil organic C
1	992.7 ± 187.3	41.3 ± 1.2	47.9 ± 3.8	4.3 ± 0.6	6.5 ± 2.0
2	869.0 ± 303.1	36.8 ± 11.9	38.7 ± 10.0	4.5 ± 0.3	7.5 ± 0.9
4	829.0 ± 299.2	33.6 ± 14.0	39.3 ± 8.2	3.4 ± 0.1	7.1 ± 1.1
8	801.5 ± 274.3	32.9 ± 5.0	36.7 ± 1.2	4.0 ± 1.9	7.1 ± 3.0
15	1165.5 ± 75.0	33.6 ± 23.7	69.7 ± 12.4	7.9 ± 1.00	6.1 ± 1.7
22	917.2 ± 332.2	31.9 ± 20.2	49.3 ± 2.7	2.8 ± 0.1	8.4 ± 5.6
32	699.3 ± 226.3	29.0 ± 8.0	30.9 ± 6.0	3.4 ± 0.7	7.3 ± 0.9

Table 2 Destination of newly fixed C in the plant-soil system at the end of the chase period, and estimated annual C flux to different C pools

Destination of fixed C	Amount of labelled C (mg m^{-2})	Partitioning of ^{13}C (%)		Estimated annual C flux (kg ha^{-2})
Total assimilation	993	100.0		8377
Total loss*	293	29.6		2420
Shoots	287	28.9		2476
Allocated to belowground	583	58.7	100.0	4924
Roots	340	34.2	58.3	2868
Loss from roots†	171	17.2	29.2	1443
Soil	73	7.3	12.5	613

*Calculated as the difference between total assimilated C and total C remaining in the system on the 32nd day.
†Including root respiration and microbial respiration of rhizodeposits.

et al., 2003; Kaštovská & Šantrùèková, 2007). Despite the spatial heterogeneity, the ^{13}C flow through the plant–soil system showed a similar translocation tendency during the chase period.

Shoot ^{13}C enrichment

The ^{13}C fixed by shoots has several destinations. It may be emitted through respiration, used in production of new shoot growth, temporarily stored (e.g. in starch), allocated into roots or used by soil organisms and then released into the atmosphere by respiration. The proportion of carbon allocated to all destinations totaled 28.4% of newly fixed ^{13}C within the first 12 h from 18:00 to 06:00 hours in the current study. This percentage is consistent with a previously reported observation (Butler *et al*., 2004). During the next day, new photosynthate was added to support root allocation and shoot respiration, and part of the ^{13}C was already incorporated in the shoot structure. Therefore, during the next daytime period, loss of pulse-derived ^{13}C from shoots was less than during the previous night. Loss or export of recently fixed carbon from shoots was 36.7% within the first 24 h, which is within the range of 32–51% loss reported for an upland grassland (Johnson *et al*., 2002a), but much lower than the value of 77% reported after two sequential pulse-labelings (Ostel *et al*., 2000). The total extra ^{13}C in shoots immediately after labeling provided the best available estimate of ^{13}C assimilation during the pulse. However, it was still too late to gain the exact initial ^{13}C content in shoots when chambers were removed about 6 hours after introducing $^{13}CO_2$ for 40 min. Since some ^{13}C had been respired or allocated to belowground within this period of time, the rate of carbon loss on the first day was underestimated in this study. Besides, shoot $\delta^{13}C$ value remained stable at around 200‰ for 4 days after labeling, probably indicating that a part of the recently fixed carbon was transformed into more stable organic carbon as the structural carbon in plant tissue.

A total fraction of 55.5% of the fixed ^{13}C was exported from shoots during the 32 days of the experiment, which was lower than the values of 67% and 76% within a 6-day chase period reported by Hill *et al*. (2007) and by Kaštovská & Šantrùèková (2007), respectively. However, this was within the wide range ca. 30–90% esti-

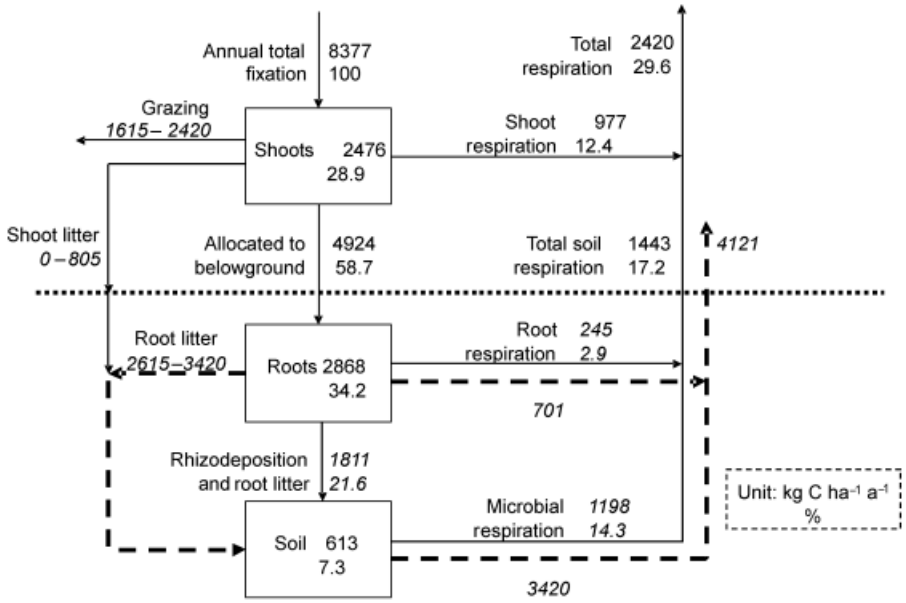

Fig. 4 Schematic diagram of annual C partitioning in diverse C pools in the *K. humilis* meadow plant–soil system representing the annual carbon fluxes. The absolute estimates (upper numbers in Kg C ha^{-1}) and their percentage (bottom numbers) of the total annual C fixation are indicated. Solid lines indicate C fixed in current year and dash lines indicate previously stored C. Numbers in italics are cited or recalculated from Cao *et al*. (2004), Du *et al*. (2007) and (Zhang *et al*., 2009). The value of 2615–3420 kg C ha^{-1} a^{-1} for root litter is the minimum value required to balance total soil loss through belowground respiration. The amount of shoot C removed or returned to the soil is determined by grazing intensity.

mated for grasses (Colvill & Marshall, 1981; Baxter & Farrar, 1999; Dilkes *et al*., 2004). The fraction of ^{13}C exported would be slightly overestimated because of shoot respiration losses of $^{13}CO_2$ occurring concurrently with partitioning, whereas it would be underestimated because part of the ^{13}C was exported but not considered during the labeling period. The main proportion of assimilated transport (ca. 66.1% of total export) from shoots occurred during the first day, which was in accordance with the findings of Butler *et al*. (2004) and Leake *et al*. (2006), who report that 31–70% of fixed carbon was lost from shoots within 24 h.

Root ^{13}C enrichment

Significant ^{13}C enrichment in roots was detectable immediately after a 4 h labeling and reached a peak between 24 and 48 h (Ostel *et al*., 2000; Johnson *et al*., 2002a). Some other studies showed that ^{13}C flux to belowground pools peaked within hours after labeling (Kuzyakov & Domanski, 2000). The speed of carbon allocation of current assimilation to belowground pools in these studies emphasizes the potential importance of newly fixed carbon for rhizodeposition. However, ^{13}C enrichment in roots was highest on day 15 after labeling in this study, which is consistent with several previous studies where at% excess ^{13}C took several weeks to reach its highest concentrations in roots (Ostel *et al*., 2003; Staddon *et al*., 2003; Rangel-Castro *et al*., 2004). The mean ^{13}C at% excess in roots was typically much lower than that in shoots, usually by more than an order of magnitude. Shoots often had a clear ^{13}C enrichment peak which was then followed by a gradual decline. A similar, but time-lagged response was seen in roots. The decrease in $\delta^{13}C$ value was proportionately slower in roots than shoots after reaching their crest values.

Root sampling for ^{13}C analysis was extremely laborious, as a significant proportion of the root mass comprises old roots, which may be living or dead. Distinguishing and separating the two fractions was a nontrivial process. In this study, roots were separated by color (black roots were defined as dead roots and others were defined as living roots). The ^{13}C at% excess of dead roots was significantly lower than that of living roots, but was significantly higher than unlabeled roots. This indicated that it was impossible to completely separate living and dead roots only based on visual color. The variability in various studies may be partly explained by different root sampling depths, the manner in which roots were extracted and sorted from soil, and the decision to discard black roots as dead roots in ^{13}C analysis.

The amount of ^{13}C allocated into roots was estimated by assuming that root biomass remained unchanged

during the whole chase period. It is important to recognize that the above method did not provide a complete measure of carbon allocation to roots since a substantial part of carbon was lost rapidly as respiration, and passed directly into the soil as rhizodeposition.

The highest proportion of fixed ^{13}C was always allocated to roots in the plant–soil system in the current experiment (Table 1). This is inconsistent with most previous studies (Saggar *et al*., 1999; Kaštovská & Šantrùèková, 2007). Labeled ^{14}C incorporated in the roots of *L. perenne* is ca. 2–29% (Kuzyakov *et al*., 1999, 2001) and in other perennial plants such as *Festuca arundinacea* and *F. pratensis* is ca. 2.5–28% (Johansson, 1991, 1992; Cheng *et al*., 1994). These figures are all lower than the value of 34.2% reserved in roots in this study. The cause of this difference may lie in two respects. Firstly, as perennial plants maintain roots throughout the year, they are likely to invest more of their productivity in roots than annuals. For instance, native grassland (*Agropyron koeleria*) in Canada has been reported to transfer 34.5–54.1% of assimilated carbon to roots (Warembourg & Paul, 1977). Secondly, plants invest more carbon to belowground parts in response to a relative shortage of any essential soil resource in order to keep an above- and belowground functional balance (Clement *et al*., 1978). For example, a greater proportion of assimilated carbon was partitioned to belowground under low soil P conditions (Saggar *et al*., 1997, 1999; Stewart & Metherell, 1999) and a lower proportion was partitioned in N fertilized plants (Kuzyakov *et al*., 2002). The alpine meadow in this study was poor in soil available N, mainly due to low temperature (Zhang & Cao, 1999). Vegetation in alpine and polar regions characterized by low temperatures are also characterized by a high root/shoot ratio (IPCC, 2007), indicating a high carbon allocation to belowground pools in these ecosystems. The *K. humilis* alpine meadow studied has a much higher root/shoot ratio than the average reported for grasslands by the IPCC (IPCC, 2007).

From a physiological perspective, root/shoot ratios have been interpreted as reflecting the differential investment of photosynthates between the aboveground and belowground organs (Titlyanova *et al*., 1999). The range of root/shoot ratio across all vegetation types varied by two orders of magnitude, with a much higher ratio in cold temperate grassland (Titlyanova *et al*., 1999; Yang *et al*., 2009). Photoassimilates partitioning pattern in this study supported the hypothesis that high root/shoot ratio in this alpine grassland was resulted from high proportion of carbon allocated to roots, but not from less carbon loss through soil respiration. Soil respiration accounted for about 17% of total assimilated carbon (Fig. 4), which was similar to other ecosystems (Warembourg & Estelrich, 2000). Percentage of newly fixed carbon transferred to belowground was also high as compared with many herbaceous species (Warembourg & Paul, 1977; Mehrag & Killham, 1990; Warembourg *et al*., 1990; Kuzyakov *et al*., 1999, 2001).

The higher allocation of newly fixed carbon to root but not soil suggested that it was root turnover but not rhizodeposition that controlled carbon flow into soil through plant roots in this ecosystem. Therefore, soil microbial activities and nutrient turnover may be negatively impacted by limited carbon source. Consequently, litter decomposition rate would be low despite of large amount of annual dead root input, in accordance with the high soil organic matter content (Yang *et al*., 2008). Since root turnover and soil organic matter decomposition may have different sensitivities to climate change and grazing (Smit & Heuvelink, 2007; Luo *et al*., 2009), a dominant human activity in the region, this carbon allocation pattern may have a profound effect on future response of carbon cycling to climate change and human activities.

Bulk soil ^{13}C enrichment

The extent of bulk soil enrichment with ^{13}C after pulse-labeling was very low but was detectable in some studies, ranging from 0.0015 to 0.0027 at% (Staddon *et al*., 2003; Rangel-Castro *et al*., 2004). We measured 0.001041 at% excess 1 day after labeling, and a peak of 0.001342% on day 22. Based on soil carbon content (typically 4–6% in the soil) and soil bulk density (approximately 0.6 g cm^{-3}), bulk soil ^{13}C enrichment was calculated to range from 61.15 to 83.58 mg m^{-2} within the top 20 cm soil profile, though the soil samples contained some very fine root materials which may have resulted in overestimation of the amount of soil derived ^{13}C.

Total carbon yield

Allocation of ^{13}C to different pools in the plant is considered to be completed when the metabolic components in plants are depleted of ^{13}C and the rate of transfer between plant and soil reaches a steady-state. The partitioning of ^{13}C was almost completed in this ecosystem within 4 days after labeling. Thus, measuring ^{13}C for up to 32 days after labeling should have left a long enough period of time for a steady-state to be reached. During the 32-day chase period, about 59% of the total assimilated carbon was allocated to belowground, in which 58.3% was recovered in root biomass, 30.6% was lost due to root respiration and rhizodeposition from roots, and 12.5% was transferred into soil, including in soil microorganisms or soil organic matter

(Table 2). The partitioning pattern among belowground parts was similar to cereals and grasses as reviewed by Kuzyakov & Domanski (2000). The rate of assimilated carbon transferred to belowground was higher than that in *L. perenne*, ranging from 5% to 39% at different growing stage (Mehrag & Killham, 1990; Kuzyakov *et al*., 1999, 2001), except at early growing stage in a field scale experiment which a high fraction of 67% was reported (Mehrag & Killham, 1990). This proportion was also comparable to some other pasture plants like *Bromus madritensis*, *B. erectus*, and *A. Koeleria* (Warembourg & Paul, 1977; Warembourg *et al*., 1990).

It is important to use a proper index to evaluate the carbon balance and carbon turnover in soils. We propose here to use ^{13}C content (mg C m^{-2}) instead of using the ^{13}C fraction (%). The annual amounts of carbon assimilated were estimated using Eqn (5) below, on the assumption that photosynthate allocation reached a steady state on the 32nd day after labeling. The estimation also assumed that carbon partitioning of vegetation was near to the annual average during the middle of the growth season. This is based on the fact that more fixed carbon was allocated to aboveground for fast shoot proliferation in the early growing season, and more carbon was translocated to belowground for storage in the late growing season. The assumption was in accordance with previous studies that vegetation carbon partitioning was near to the annual average at mid-growing season (Warembourg & Paul, 1977; Stewart & Metherell, 1999).

$$\text{Estimated assimilated C} = (A_{\text{shoot}} \times C_{\text{shoot}})/{}^{13}C_{\text{shoot}}, \quad (5)$$

where estimated assimilated carbon is annual carbon fixation (kg C ha^{-1}); A_{shoot} is annual shoot growth expressed by maximum aboveground biomass (kg C ha^{-1}); C_{shoot} denotes shoot carbon concentration (%); and $^{13}C_{\text{shoot}}$ is the percentage of net assimilated ^{13}C remaining in the shoots on day 32. The estimated assimilated carbon was then partitioned into the plant–soil carbon pools based on the ^{13}C distribution on day 32.

Under the conditions mentioned above, we estimated the total annual carbon flow into belowground pools in this alpine meadow to be about 4924 kg C ha^{-1} (Table 2). Using the respiration-biomass regression method, Zhang *et al*. (2009) separated ecosystem respiration into aboveground and total soil respiration, which had an individual contribution of 0.42 and 0.58, respectively. These values matched the contributions of 0.40 and 0.60 identified in this study (Fig. 4), despite the fact that both newly fixed carbon and stored carbon were involved in the measured respiration. Adopting the relative contribution of root and soil microbial respiration to total soil respiration in the same article (Zhang *et al*., 2009), we calculated that roots lost only 2.9% of annual fixed carbon through autotrophic respiration, while it transferred seven times more carbon to the soil by rhizodeposition (Fig. 4), which is similar to results of labeling experiments in a U4d (*Festuca-Agrostis-Galium-Luzula multiflora-Rhytidiadelphus loreus* subcommunity) grassland at Sourhope (Leake *et al*., 2006), but much higher than the fraction of 7–12% reported in warmer climate regions for a *L.* perenne and *Trifolium repens* grassland (Kaštovská & Šantrùèková, 2007), *L. perenne* plants (Butler *et al*., 2004), *B. erectus* plants (Warembourg & Estelrich, 2000), and *Zea mays* plants (Haller & Stolp, 1985; Jones & Darrah, 1993).

In annual assimilated carbon, 2.476 t ha^{-1} remained aboveground in shoot, 2.868 t ha^{-1} in root and 0.613 t ha^{-1} in soil (Fig. 4). Carbon storage was reported to be 11.1 and 78.4 t ha^{-1} in roots and soil (0–20 cm) in this grassland (Zhao, 2009). Therefore, carbon turnover rate in root and soil was about 25.8% and 0.78% per year, corresponding to mean residue time of 3.9and 128 years, assuming the humification coefficient for the root-derived carbon is 10% (Kuzyakov *et al*., 2001) and soil carbon stock approximated constant.

The measured maximum aboveground biomass of this vegetation is reported to be 550 g DW m^{-2} (Du *et al*., 2007). A previous investigation using a closed chamber method indicated that total soil respiration in this ecosystem lost 5564 kg C ha^{-1} annually (Cao *et al*., 2004). It appears that the direct carbon flow into belowground carbon pools through living plant carbon translocation was insufficient to balance the respiratory carbon loss. The alpine meadow studied was used as a winter pasture. Under high grazing intensity and with little return of aboveground litter to soil, the plant–soil system may become a weak source of atmospheric CO_2 (Fig. 4). Without litter input, the belowground carbon pool would have a net loss of 643 kg C ha^{-1} a^{-1}. Under a light grazing regime (e.g. 3 sheep unit ha^{-1}), aboveground litter return (94 g DW m^{-2}) transformed the system into a weak carbon sink, about 162 kg C ha^{-1} a^{-1}. Calculating using the relative ratio of autotrophic and heterotrophic respiration from (Zhang *et al*., 2009), about 701 kg C ha^{-1} a^{-1} was lost through root respiration of stored carbon (Fig. 4). To balance soil carbon loss, about 2615–3420 kg C ha^{-1} a^{-1} would have to be transferred to soil through root litter. This corresponded to a root carbon turnover rate of 33–40% per year based on our measured root biomass, and which is in accordance with findings from other grasslands of similar temperature (Gill & Jackson, 2000). This indicates that the large root carbon storage may buffer soil carbon loss resulting from variation in aboveground litter input. Yak grazing

decreased both above- and belowground plant biomass, with more reduction under higher grazing intensity in the alpine meadow on Tibetan Plateau (Dong *et al*., 2005). Grazing also altered soil CO_2 emission rate of alpine meadow (Cao *et al*., 2004). Thus, grazing intensity had an essential role in determining the carbon balance of the alpine meadow ecosystem.

Uncertainty in estimation of carbon allocation in the ^{13}C pulse labeling method

Stable isotope pulse labeling had been widely used to quantify carbon flows in plant–soil systems. Yet, there are still several inherent sources of uncertainty in this method. Firstly, carbon allocation was calculated based on the amount of initially fixed ^{13}C at the end of labeling. This value was difficult to measure directly, and has been approximated by ^{13}C recovered in shoots immediately after labeling (Saggar *et al*., 1997; Butler *et al*., 2004; Leake *et al*., 2006), or by summation of ^{13}C in shoots, roots, and soil immediately after pulse-labeling (Kuzyakov *et al*., 1999; Hill *et al*., 2007; Kaštovská & Šantrùèková, 2007). No matter which method is used, solely basing the estimate on ^{13}C enrichment from pulse-labeling leads to an underestimation of the total amount of carbon fixed and translocated to carbon pools, due to fixation of $^{12}CO_2$ that was already in the chamber before $^{13}CO_2$ supply and which is continuously emanated through plant and soil respiration during labeling.

Secondly, as observed in our study, the amount of ^{13}C in shoots immediately after labeling was even less than the total amount in the plant–soil system 1 day after labeling, implying that part of recently fixed carbon was translocated to belowground during labeling. This suggests that using shoot ^{13}C content alone would underestimate the initial amount of ^{13}C fixation. Particular caution should be taken in pulse labeling over longer periods, or in vegetation with rapid carbon allocation (Saggar *et al*., 1997; Butler *et al*., 2004; Leake *et al*., 2006).

Thirdly, extrapolation of the results of a single pulse-labeling and chase study provides an economic way to estimate whole season or annual carbon allocation. However, this suffers from uncertainty due to variation in the seasonal allocation pattern. The season of greatest carbon allocation to roots has been reported to be spring/autumn or spring for *L. perenne*, and summer for a Canadian native grassland (Parsons & Robson, 1980; Colvill & Marshall, 1984), and some other studies reported that carbon partitioning of vegetation was near to the annual average during the middle of the growing season (Warembourg & Paul, 1977; Warembourg *et al*., 1990).

These uncertainties were partly acknowledged but the degree of deviation was not quantified in all these studies. More detailed studies are needed to quantify and reduce the uncertainties associated with the pulse labeling method.

Conclusions

Use of ^{13}C as a tracer allowed us to estimate the approximate proportions of recently fixed carbon allocated from shoots to roots and soil under unmodified field conditions (Table 2). About 36.7% of newly assimilated carbon (equal to 66.1% of the total amount of ^{13}C exported from shoots) was lost within the first 24 h, indicating an intensive translocation of ^{13}C-labled novel photosynthate out of the shoots of alpine meadow plants. 58.3% of total assimilated carbon – much higher than the proportion in perennial grasses like ryegrass – was transferred to belowground pools. Living roots accounted for the highest proportion of total ^{13}C in the plant–soil system during the 32 days of the study. Comparison of estimated carbon flux into belowground pools and reported soil respiration carbon loss indicated that the alpine meadow ecosystem was sensitive to grazing intensity in terms of carbon budget.

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